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ORIGINAL ARTICLE

A novel bacterial carboxylesterase identified in a metagenome derived-clone from Brazilian mangrove sediments

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Abstract

A functional screening of 1152 clones from a plasmid library constructed with DNA extracted from Brazilian mangrove sediments revealed 3 positive clones for ester-hydrolyzing enzymes, or about one lipolytic gene per 1.2 Mb DNA, which corroborates the idea that oil-contaminated mangroves are a good source of novel microbial lipases/esterases. The partial sequence of the clone LipG7 (1179 bp) showed 30.2% of predicted structure identity with a known esterase, confirming LipG7 as a new member of family VIII esterases. LigG7 shared 80% sequence identity with 1,4-butanediol diacrylate esterase from the Gammaprotebacteria *Porticoccus hydrocarbonoclasticus*, suggesting it belongs to the Porticoccaceae family. LipG7 was heterologously expressed in *Escherichia coli* Rosetta-Gami DE3; the purified recombinant enzyme exhibited a predicted molecular weight of 45.2 kDa and exceptional activity towards 4-nitrophenyl butyrate, compared with other recombinant esterases, highlighting its enormous potential for biological applications.

Keywords Metagenomic library · 1.4-Butanediol diacrylate esterase · Family VIII · *Porticoccus* · Heterologous expression

Introduction

Microorganisms are the main source of industrial enzymes due to their high catalytic efficiency, fast growth in low cost media, high production yields, stability and ease of genetic manipulation [1]. Despite the large number of studies exploring the enzymatic potential of microorganisms, it is noteworthy that currently only 1% of the environmental microorganisms can be cultured in the laboratory, leaving the vast majority of the microbial resources untapped [2, 3]. Metagenomics, a culture-independent approach, circumvents this limitation by making the total DNA from environmental samples easily accessible through sequencing or cloning into a host cell followed by screening by either sequence- or activity-driven procedures [3]. Several studies have confirmed the power of metagenomics for the discovery of new microbial enzymes from natural resources [4–7].

Esterases and lipases are the most studied enzymes due to their broad industrial application in food, detergent, pharmaceutical, leather, textile, cosmetic, biofuels production and paper industries [8–10]. Their vast range of applications relates to their stability in organic solvents, high substrate specificity, stereoselectivity, regioselectivity and activity in the absence of cofactors [11]. Lipolytic enzymes catalyze ester hydrolysis, ester synthesis and transesterification reactions. The triad of these enzymes is composed of nucleophilic residues (Ser, Cys or Asp), catalytic acidic residues (Asp or Glu) and a conserved histidine [12]. While lipases (EC 3.1.1.3) catalyze ester bonds of large water-insoluble acylglycerols (> 10 carbons), carboxylesterases (EC 3.1.1.1) exert catalytic action on water-soluble short-chain acylglycerols (< 10 carbons) [12, 13].

At the current pace, the global enzyme market is projected to reach $ 7 billion by 2023 [14]. This will keep stimulating the prospection of genetic resources from nature.

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Mangroves are coastal ecosystems with unique ecological dynamics due to periodic tidal flooding, which imposes variations in sediment salinity [15]. Variations in salinity associated with the abundance of organic matter and the propensity of sediments to accumulate pollutants favors the development of a plethora of microorganisms with extremely broad metabolic capabilities [16, 17], representing a potential source of new enzymes.

Thus, the aim of this study was to prospect the genetic resource of mangrove sediments from the semiarid region of Brazil by screening a metagenomic clone-library searching for novel ester-hydrolyzing enzymes.

Materials and methods

Sediment sampling

To access typical mangrove microhabitats, three sites were sampled in the Jaguaribe mangrove, Northestern Brazil. Site S1 (S 04°26′43.9 W 037°46′59.9) was the closest to the sea in an area without vegetation; the second site S2 (S 04°26′45.1 W 037°47′00.6) was located in an area of *Rhizophora mangle* forest; and the third site S3 (S 04°26′46.8 W 037°47′00.7) was located in a region covered by *Avicennia schaueriana* (Fig. 1). Sediment samples were collected during spring tides (amplitude 0.1) at a depth of 0–10 cm using a 10 cm diameter cylindrical sampler. Five replicates within each region were randomly collected and pooled to form a representative composite sample of each habitat.

Metagenomic library construction

The construction of the metagenomic library was performed according to Uchiyama et al. [18] with adaptations. Total DNA from pooled sediment samples was extracted using PowerMax Soil DNA Extraction kit (MoBIO, Carlsbad, CA, USA) according to the manufacturer's instructions. Next, a total of 1.5 µg genomic DNA was digested with the endonuclease *Eco*RV (Thermo Fisher Scientific, Waltham, MA, USA) and inserts ranging from 1 to 8 Kb were ligated into the vector pJET1.2 blunt (Fermentas, Waltham, MA, USA) for subsequent transformation in *E. coli* TOP 10F' (Thermo Fisher Scientific, Waltham, MA, USA). Transformed clones were stored in LB broth (10 g/L peptone, 5 g/L yeast extract and 5 g/L NaCl) supplemented with 10% v/v glycerol at − 80 °C until use.

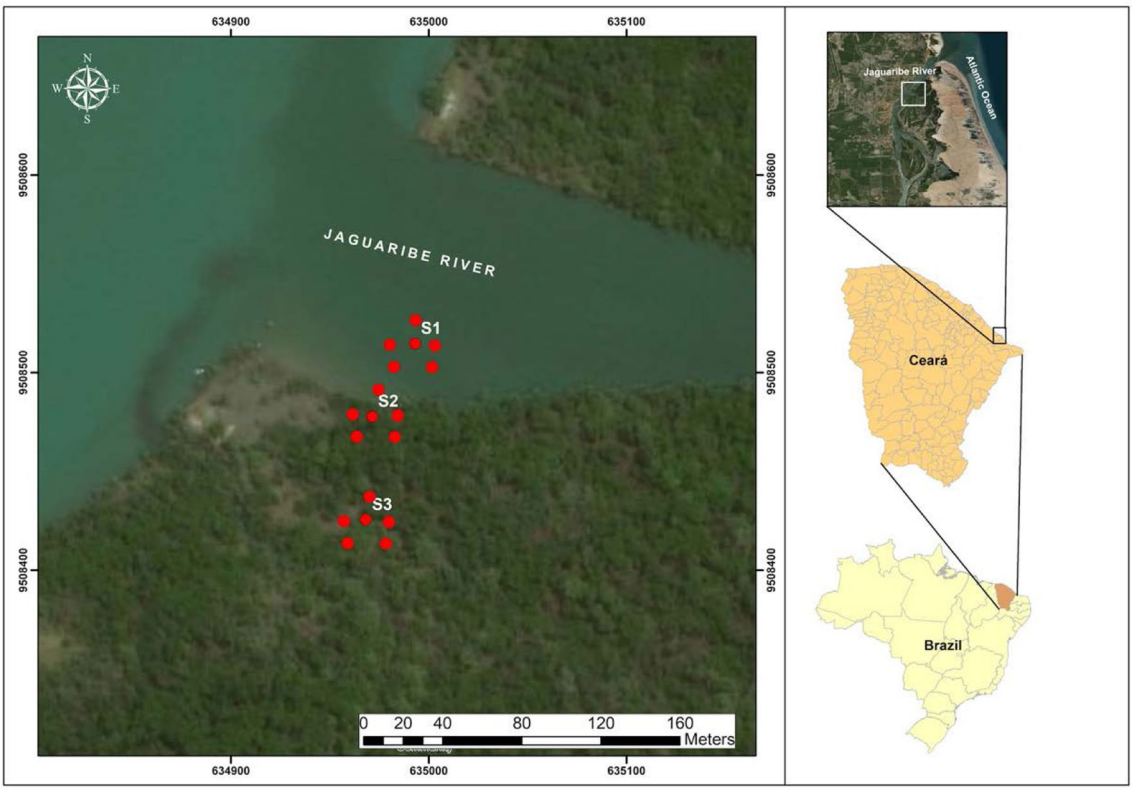

Fig. 1 Study area in the Jaguaribe River estuary, Ceará, Brazil and the location of sampling sites S1 (S 04°26′43.9″W 037°46′59.9″), S2 (S 04°26′45.1″W 037°47′00.6″) and S3 (S 04°26′46.8″W 037°47′00.7″) in the mangrove habitats

Screening for esterase activity in metagenomic library

For enzyme screening, clones were initially reactivated in 96-well plates containing 1.0 mL of LB broth supplemented with ampicillin (Sigma-Aldrich, San Luis, MO, USA) (100 µg/mL) and incubated at 37 °C for 24 h with shaking at 150 rpm. Clones were replicated using a 96-pin array onto LB agar plates containing 1% v/v tributyrin (Himedia, Mumbai, Maharashtra, India) and incubated at 37 °C for 48 h. The appearance of a hydrolysis halo around the colonies was considered a positive indication of esterase activity.

Sequencing analysis of esterase genes

Plasmid DNA from clones exhibiting esterase activity was extracted following Birnboim method [19] and analyzed by electrophoresis on a 1% (w/v) agarose gel stained with SYBR™ safe DNA gel stain (Invitrogen, Carlsbad, CA, USA).

Plasmids were sequenced at Macrogen company (www.dna.macrogen.com) by primer walking starting with the forward primer pJET1_2F 5′-CGACTCACTATAGGGAGA GCGGC-3′ and reverse primer pJET1_2R 5′-AAGAACATC GATTTTCCATGGCAG-3′ using the ABI PRISM Bigdye™ terminator cycle sequencing kit (Applied Biosystems, Foster City, CA, USA). Partial sequences were used to generate consensus sequences with CodonCode Aligner 5.1.1 (www.condoncode.com).

Sequences were analyzed and compared with the non-redundant proteins from the National Center for Biotechnology Information—NCBI (www.ncbi.nlm.nih.gov/) using the Basic Local Alignment Search Tool – BLASTx tool [20] for protein identity and amino acid sequence motif searches. Multiple sequence alignment was performed using the ClustalW program [21] with related enzymes to analyze conserved patterns. Physical and chemical parameters were analyzed using ProtParam tools (https://web.expasy.org/protparam/).

Sequence alignment and homology modeling

For construction of the phylogenetic tree, multiple sequences were aligned using the program ClustalW [21] and phylogenetic analysis was performed using Geneious® 9.1.5 software (https://www.geneious.com) [22] and the Neighbour-Joining [23] method with bootstrap based on 1000 replications. Tamura and Nei [24] was used for estimating pairwise evolutionary distances. The phylogenetic tree was edited on MEGA7 for bigger datasets [25]. Phylogenetic tree was constructed using 17 enzymes related sequences chosen based on the BLASTx results.

The target sequence was submitted to the SWISS-MODEL server (automated comparative protein modeling sever) (https://swissmodel.expasy.org/) for comparative structural modeling [26]. As the structure of LipG7 presented 30.2% identity with the crystal structure of the trans-esterase from of *Aspergillus terreus* (PDB 4LCL:A), it was used as a template for homology modeling.

Subcloning of LipG7

Subcloning of LipG7, the only gene with homology to known lipolytic enzymes, was performed into pET302/NT, a vector containing a lac promoter and a N-terminal 6×His tag. Gene amplification by PCR was performed using primer pair LipG7_F 5′-AAA GAA TTC AGA GAA TCT TTA TTT TCA GGG CAT GAG CGA CTC ACT GCG TCA GG-3′ and LipG7_R 5′-AAC TCG AGT CAT TTC AGG TGA CGA TAG ATT GCC G-3′ (Integrated DNA Technologies, Coralville, IA, USA). The reaction was performed using a thermocycler Mastecycler® (Eppendorf, Hamburg, HH, Germany) in a final volume of 20 µL containing 100 ng of template DNA, Go Taq Buffer 1× (Promega, Madison, WI, USA), 3 mM $MgCl_2$, 0.2 mM of each dNTP, 5 µM of primer and 1.0 unit of GoTaq DNA Polymerase (Promega, USA). Cycling conditions were as follows: one initial denaturation step of 5 min at 94 °C followed by 30 cycles of 1 min at 94 °C, 1 min at 61.8 °C and 1 min at 72 °C. PCR products were analyzed by electrophoresis on 1% (w/v) agarose gel, visualized with SYBR™ safe DNA stain (Invitrogen, USA) and purified using the Wizard™ SV gel and PCR clean-up system (Promega, USA). PCR product and pET302/NT vector were digested with *Eco*RI and *Xho*I (Thermo fisher Scientific, Waltham, MA, USA) and ligated overnight at 16 °C at an insert to vector ratio of 3:1. Plasmids were inserted into *E. coli* Rosetta-Gami DE3 (Merck Millipore, Burlington, MA, USA) by electroporation (BTX ECM 399, Holliston, MA, USA) which were subsequently plated on LB agar supplemented with ampicillin (100 µg/mL). Colonies were picked and submitted to single colony PCR using the conditions described above to confirm the presence of the insert and subsequently grown in LB broth for storage at − 80 °C in a glycerol stock until use.

Protein expression and purification

Protein expression was performed according to Sambrook [27] with adaptations. A pre-culture of transformed cells was grown overnight in 15 mL LB supplemented with ampicillin (100 µg/mL) at 37 °C and 200 rpm. Next, 4 mL of the pre-culture were transferred to 400 mL LB broth ampicillin (100 µg/mL) and grown at 37 °C, 200 rpm to an optical

density (OD_{600nm}) of 0.6. Induction of enzyme expression was tested under three isopropyl-β-D-thiogalactoside (IPTG) concentrations: 0.1 mM, 0.5 mM and 1 mM, and three temperatures (20 °C, 30 °C and 37 °C). After 3 h induction, cells were centrifuged, sonicated (Qsonica Q125, Newtown, CT, USA) on ice 2 times for 3 s on and 3 s off for 5 min, 40% amplitude and centrifuged at 15,000×*g*, 4 °C for 45 min. Washing of inclusion bodies was performed three times in 100 mM Tris–HCl buffer, 5 mM EDTA, 5 mM DTT, 2 M Urea and 2% Triton X-100 and 2 times in 100 mM Tris–HCl buffer followed by centrifugation for 30 min, 15,000×*g* at 4 °C. After each washing step, aliquots were kept for quality control. Inclusion bodies were resolubilized in 50 mM Tris–HCl pH 8.0 and 3 M guanidine hydrochloride buffer, incubated 30 min at room temperature and centrifuged for 8 min, 15,000×*g* at 4 °C.

Purification of the protein was done by affinity chromatography using Ni-Sepharose 6 Fast Flow (GE healthcare life sciences, Chicago, IL, USA). The column was equilibrated with 50 mM Tris–HCl and 100 mM NaCl buffer and loaded with the sample containing the enzyme. Washing of the column was performed with 50 mM Tris–HCl buffer, 100 mM NaCl and 6 mM imidazole. Matrix-bound enzyme was eluted by adding 50 mM Tris–HCl buffer, 100 mM NaCl and imidazole varying from 50 to 200 mM. The eluted fraction was extensively dialyzed against distilled water at 4 °C to ensure complete imidazole removal. The presence of the target protein was detected by SDS-PAGE electrophoresis (15%) after IPTG induction, treatment of inclusion bodies and chromatography purification. The final enzyme concentration was determined using an extinction coefficient of 1.37 mL/mg/cm at 280 nm, which was deduced from the amino acid sequence.

Evaluation of specific activity

The specific activity of the esterase was analyzed using the substrate 4-nitrophenyl butyrate (Sigma-Aldrich, San Luis, MO, USA) in 96-wells microplates. The enzyme (2 µg) was incubated with 1.0 mM substrate in 50 mM phosphate buffer, pH 7.2 at 30 °C for 30 min in a total volume of 260 µL. The reaction was quenched by chilling on ice and the amount of released 4-nitrophenyl was determined by an absorption increase at 410 nm against an enzyme-free blank on an Epoch microplate spectrophotometer (Biotek, Winooski, VT, USA). One unit of esterase was defined as the amount of enzyme needed to generate 1.0 µmol of 4-nitrophenyl per min under the conditions described above [7]. Assays were performed in triplicate.

Effect of the addition of divalent ions and detergents on esterase activity

The effect of metal ions, nonionic surfactants (Triton X-100 and Tween 80) and EDTA on the catalytic activity of the esterase was evaluated under the conditions described above [7]. For this, enzymatic reaction containing 1 mM of 4-nitrophenyl butyrate in 50 mM phosphate buffer pH 7.2 was determined in the presence of 0.5 mM of Mg^{2+}, Mn^{2+} or Ca^{2+}, 1% (v/v) Triton X-100, 1% (v/v) Tween 80 and 0.5 mM EDTA. The reaction was incubated at 30 °C for 30 min and subsequently analyzed in a spectrophotometer at 410 nm. For greater reliability of the results, control reactions were made for each condition. All experiments were analyzed in triplicates and the data were statistically analyzed by ANOVA and the multiple comparisons method (Tukey test) using the program GraphPad Prism version 6.01.

Results

Screening of esterase activity in metagenomic clone library

A total of 1152 clones were obtained from metagenomic DNA of mangrove sediments with an average insert DNA size of 3 kb, representing 3.4 Mb of the total DNA. Functional screening of ester-hydrolyzing enzymes based on the hydrolytic activity of emulsified tributyrin revealed three positive clones, hereafter named as LipG6, LipG7 and LipH7, as evidenced by a clear halo around the colonies (Fig. 2). This number of clones corresponds to

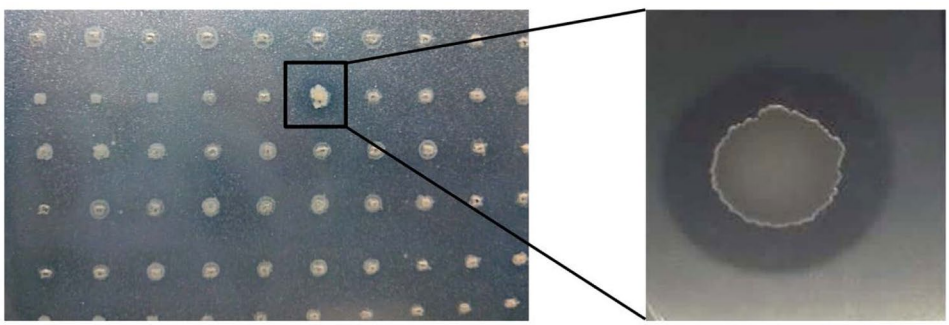

Fig. 2 Lipolytic activity of metagenomic clones demonstrated by the appearance of clear halos in tributyrin agar plates. The inset shows the activity of clone LipG7 in detail

approximately one lipolytic gene per 1.2 Mb DNA (one positive clone per 384 tested clones).

Sequencing and analysis of esterase genes

To identify the three genes possessing esterase activity, plasmid DNA was isolated from the positive clones and sequenced by primer walking. Sequencing revealed inserts of approximately 4.0, 2.9 and 0.8 kb and a GC content of 48.60, 58.72 and 56.37% for LipG6, LipG7 and LipH7, respectively. The phylogenetic relationship of the three genes with known lipolytic enzymes was analyzed performing a Blastx query against the non-redundant database from NCBI. LipG6 and LipH7 showed the closest homologies with AraC family transcriptional regulators and phosphatidyltransferases, respectively. The partial sequence of LipG7 (1179 bp) showed 80% identity with 1,4-butanediol diacrylate esterase (WP_081890792.1) from *Porticoccus hydrocarbonoclasticus*, followed by 72% identity with esterases from *Methylibium sp.* and 71% with *Parageobacillus thermoglucosidasius* (Table 1). The final LipG7 sequence was deposited in the GenBank under the accession number MH136560.

Sequence alignment and homology modeling

Analysis of the amino acid sequence of LipG7 identified it as a member of Family VIII carboxylesterases with a sequence of 392 amino acids and a predicted molecular weight of 45.2 KDa. The isolelectric point (pI) of this enzyme was 4.96.

Phylogenetic analysis based on the amino acid sequence of 17 related lipolytic enzymes grouped LipG7 with the esterase from *P. hydrocarbonoclastus* based on sequence similarity (Fig. 3).

Table 1 Top hits obtained for protein–protein BLASTx of cloned esterase genes from metagenomic library of mangrove sediments

Clone	Description	Species	Query cover (%)	E. value	Identity (%)	Accession number
LIPG7	1,4-Butanediol diacrylate esterase	*Porticoccus hydrocarbonoclasticus*	99	0.0	80	WP081890792.1
	1,4-Butanediol diacrylate esterase	*Methylibium petroleiphilum*	98	0.0	72	WP011829040.1
	1,4-Butanediol diacrylate esterase	*Parageobacillus thermoglucosidasius*	98	0.0	71	WP042383693
LIPG6	AraC transcriptional regulators	*Fluoribacter bozemanae*	22	6.00E−121	62	WP058458094.1
	AraC transcriptional regulators	*Shewanella mangrovi*	20	3.00E−101	54	WP063355125.1
LIPH7	CDP-Alcohol phosphatidyltransferases	*Porticoccus hydrocarbonoclasticus*	31	2.00E−28	68	WP051921200.1
	GtrA family protein	*Porticoccus hydrocarbonoclasticus*	41	9.00E−24	62	WP081890842.1
	Inositol-3-phosphate synthase	*Porticoccus hydrocarbonoclasticus*	16	3.00E-14	79	WP036859314.1

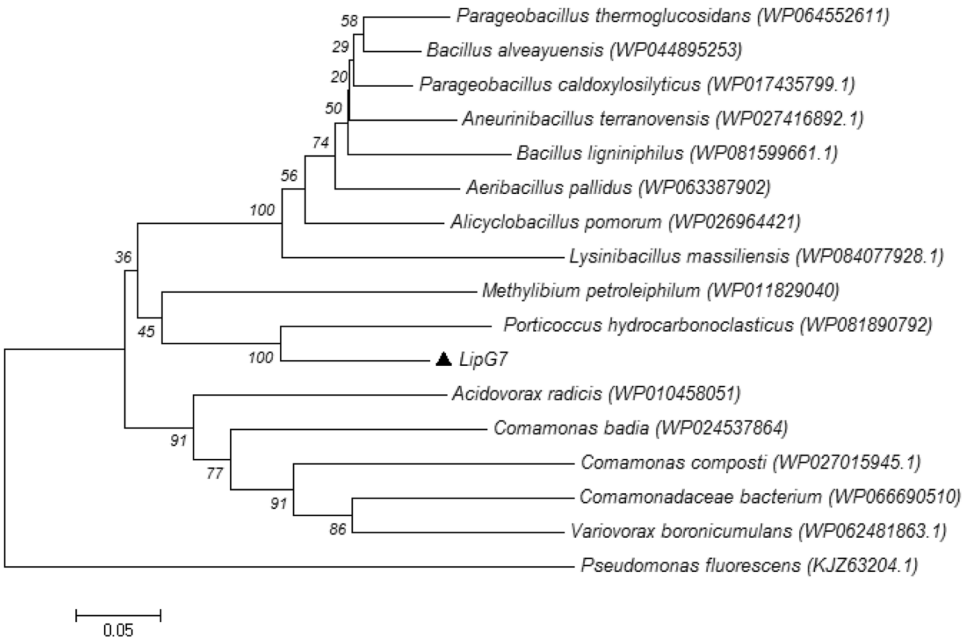

Fig. 3 Majority rule consensus tree from 17 aminoacids sequences chosen based on the BLASTx results using the neighbor-joining method with Tamura-Nei distances (NJ-TN)

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                                  ....|....|  ....|....|  ....|....|  ....|....|
                                        60          70          80          90
LipG7 (MH136560)                  EMGKDQPMTP  DTVFAIFSCT  KALTGTTLMQ  LVEEGVVSLN
Porticoccus h. (WP081890792.1)    EMGKTEPIAT  DSVFAIFSCT  KALTGTALMQ  LVEEGLVRLD
Methylibium p. (WP011829040.1)    ELGSDRPMTV  DTVMCLFSCT  KALTGAMVMQ  LVEEGLVGLD
Parageobacillus t. (WP0423836)    EFGKETAMTT  DTVFALFSTT  KAITGTALMQ  LVEEGKVKLD
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Fig. 4 Conserved regions shared by LipG7 and 1,4-butanediol diacrylate esterases from *Porticoccus hydrocarbonoclasticus*, *Methylibium petroleiphilum* and *Parageobacillus thermoglucosidasius*

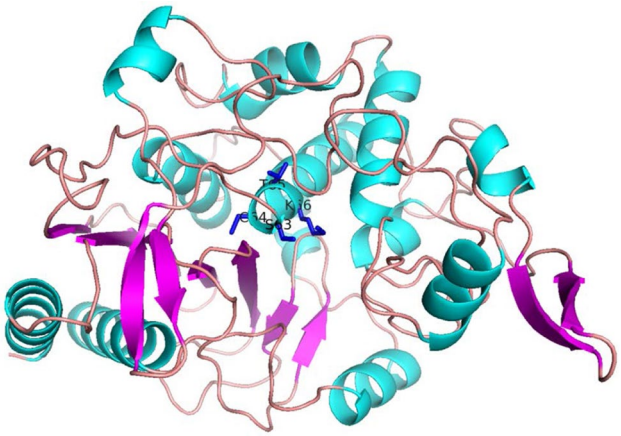

Fig. 5 Structure prediction of LipG7 by homology modeling using the transesterase from *Aspergillus terreus* (PDB 4LCL:A) as reference. The motif S_{63}-C_{64}-T_{65}-K_{66} is represented as blue sticks

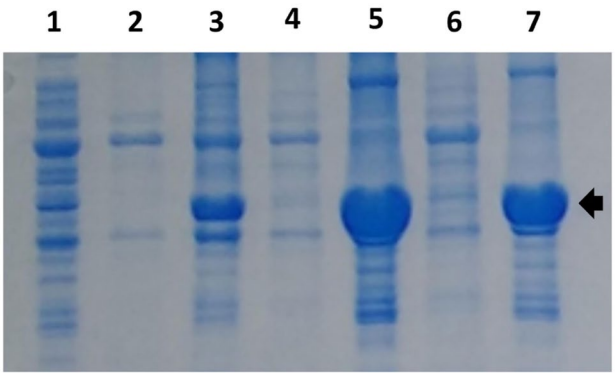

Fig. 6 SDS-PAGE of LipG7 in the soluble and insoluble fraction after cell lysis, induction in Rosetta-gami at 30 °C. (1) Non-induced; (2) fraction soluble; (3) fraction insoluble 0.1 mM IPTG; (4) fraction soluble; (5) fraction insoluble 0.5 mM de IPTG; (6) fraction soluble; (7) fraction insoluble 1 mM IPTG

LipG7 presents domains well-conserved in β-lactamases and its S-C-T-K sequence (Fig. 4) corresponds to the conserved S-x-x-K motif found in both class C β-lactamase [28] and family VIII carboxylesterases [12]. Other typical motifs related to family VIII like LxxxPGxxW, GGxG and Yxx and a variation of the motif PLGMxDTxF [29] were also detect in LipG7, confirming its classification.

Regarding its predicted structure, LipG7 presented 30.2% identity with the transesterase from *Aspergillus terreus* LovD9 [30] and its structure homology modeling suggests an α/β hydrolase fold consisting of a seven-stranded antiparallel β-sheet core interleaved by α-helices (Fig. 5).

Subcloning, heterologous expression and purification of recombinant LipG7

For overexpression in *E. coli*, LipG7 was subcloned into the pET302/NT vector under control of the *lac* promoter and fused to a 6× His tag at its N-terminus. Optimal conditions for LipG7 expression were 0.5 mM IPTG induction and 30 °C for 3 h. Recombinant LipG7 was expressed in inclusion bodies and appeared as a major band corresponding to approximately 15–20% of the total cell protein content (Fig. 6).

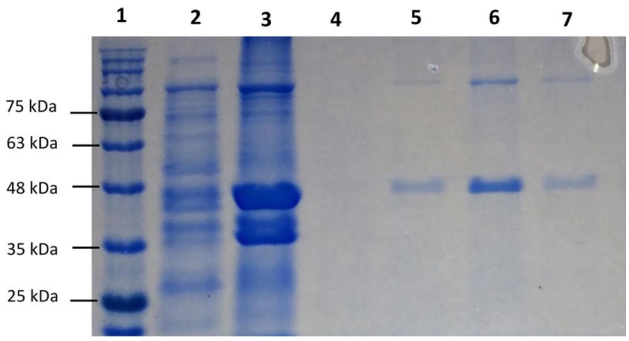

Fig. 7 SDS-PAGE showing the elution of LipG7 under imidazole gradient. (1) Molecular weight; (2) Non-induced; (3) induced; (4) Imidazole 50 mM; (5) Imidazole 100 mM; (6) Imidazole 150 mM; (7) Imidazole 200 mM

LipG7 resolubilization was performed with 3 M guanidine hydrochloride followed by removal of this chaotropic agent with protein renaturation occurring during affinity chromatography purification. SDS-PAGE confirmed LipG7 predicted molecular weight of 45.2 kDa. Elution of LipG7 from nickel affinity chromatography was optimal with 150 mM imidazole (Fig. 7). The final yield was of 6.17 mg water-soluble LipG7 per liter of bacterial culture.

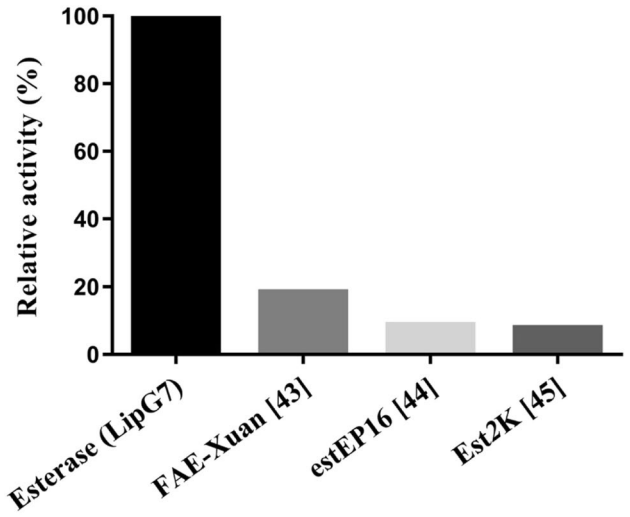

Fig. 8 Comparison of the activity of LipG7 with esterases retrieved from metagenomic library reported in other studies: Li et al. [43]; Zhu et al. [44] and Kim et al. [45]

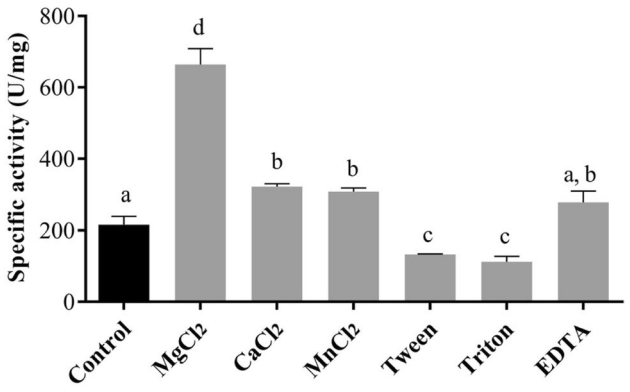

Fig. 9 Enzymatic activity of LipG7 under the influence of bivalent ions, detergents and EDTA

Specific esterase activity of LipG7

Purified LipG7 exhibited high activity against the synthetic substrate 4-nitrophenyl butyrate of 216.3 ± 16.4 U/mg at 30 °C, pH 7.2. Figure 8 compares the activity of LipG7 with other esterases retrieved from metagenomic libraries in other studies.

Effect of the addition of divalent ions and detergents on LipG7 activity

The presence of 1.0 mM Mg^{2+}, Mn^{2+} or Ca^{2+} significantly enhanced LipG7 activity, with Mg^{2+} leading to a 192% increase. Addition of 0.5 mM EDTA did not affect LipG7 while Tween-80 and TritonX-100 reduced its activity by about 50% (Fig. 9).

Discussion

Herein we report the expression of a recombinant esterase from a mangrove sediment metagenome clone-library. The amino acid sequence of this esterase, named LipG7, showed identity homology with bacterial serine hydrolase esterases from *Porticoccus hydrocarbonoclasticus* (80% identity), *Methylibium petroleiphilum* (72% identity) and *Parageobacillus thermoglucosidasius* (71% identity) (Table 1). The phylogenetic tree generated with 17 amino acid sequences based on BLASTx (Fig. 3) indicated that LipG7 was distinctly grouped with *P. hydrocarbonoclasticus*, a highly specialized hydrocarbon-degrading bacterium (Proteobacteria) isolated from marine phytoplankton [31].

The Porticoccaceae family possesses only two cultured representatives so far: *P. litoralis* isolated from the sea water in Yellow Sea, Korea [32] and *P. hydrocarbonoclasticus* isolated from the dinoflagellate *Lingulodinium polyedrum* CCAP1121/2 from Loch Creran, Scotland, by culturing the isolate in a medium containing polycyclic aromatic hydrocarbons [31]. *P. hydrocarbonoclasticus* is strictly anaerobic, rod-shaped, Gram negative, halophile and is able to use hydrocarbons as its sole carbon and energy source, while sugars cannot be used as substrates. Other metagenomic surveys have detected the presence of Porticoccaceae representatives in seawater related to oil biodegradation [33]. The 80% sequence identity shared by LipG7 and the esterase from *P. hydrocabonoclasticus* suggests that LipG7 belongs to the Porticoccaceae family.

The identification of three out of 1152 screened clones able to degrade the synthetic substrate 4-nitrophenyl butyrate with a positivity rate of 0.26% contrasts with most metagenomic screenings, that generally identify only a few positive clones among several thousands [16, 34–36]. Henne et al. [35] reported the identification of one clone with lipolytic activity out of 73,000 clones screened from a metagenomic library built from three different soil samples, corresponding to one positive clone per 473 Mb metagenomic DNA. In another study, screening of a metagenomic library built from oil contaminated soil identified one clone with lipolytic activity among 4224 clones, corresponding to one lipolytic gene for every 4.9 Mb metagenomic DNA [36]. Further, Couto et al. [16] isolated a lipase from a fosmid library from mangrove sediment containing 2400 clones with inserts around 20–30 kb, corresponding to one lipolytic gene per 60 Mb DNA.

Estuarine zones are known for their property of accumulating contaminants discharged over the river course, being a transitional storage area for these compounds. It is worth mentioning that the estuarine region of the

Jaguaribe mangrove, located nearby the sampling areas, was classified as moderate to highly contaminated with polycyclic aromatic hydrocarbons (PAHs), presenting levels as high as 3172.3 ng/g [37], besides high contamination with the pesticides DDT, α-endosulfan and heptachlor [38]. The presence of these contaminants contributes to the selection of lipolytic enzyme producers. Therefore, the identification of three positives clones for esterases from a relatively small library in the current study reinforces the potential of oil-contaminated mangroves as a reservoir of enzymes of biotechnological interest [5] as well as a methodological guide for the isolation of current non-cultivable producers [31].

LipG7 belongs to the family VIII esterase and presents homology to 1,4-butanediol diacrylate esterase and β-lactamases. Given that the family VIII esterase contains a region of high identity with β-lactamases and other penicillin-binding proteins, it is surprising that it is still poorly characterized [39]. β-Lactamases are of medical interest because they confer microbial multi-resistance to β-lactam antibiotics through hydrolysis of the β-lactam ring [40].

Furthermore, differently from other microbial esterase families in which the active serine residue is found within the G-x-S-x-G or G-D-S-L motifs [41], family VIII members present the active serine residue within the S-x-x-K motif [42]. LipG7 presents the SCTK motif, thus confirming its β-lactamase site belonging to family VIII. It was also observed that LipG7 maintains conserved the key catalytic residues Lys66, Tyr174 and Ser63 in the necessary structural position required for its activity as demonstrated by Jiménez- Osés et al. [30].

The purified recombinant enzyme, expressed in *E. coli* Rosetta-gami, exhibited optimal activity against the synthetic substrate 4-nitrophenyl butyrate of 216.3 ± 16.4 U/mg at 30 °C, pH 7.2. This activity is significantly higher than the specific activity against the same substrate reported for other esterases retrieved from metagenomic libraries [43–45], highlighting the potential of LipG7 (Fig. 8).

In general, lipolytic enzymes do not require cofactors for their catalytic activity. However, to date, several lipases and esterases have been described to possess enhanced activity in the presence of divalent cation cofactors such as Ca^{2+}, Zn^{2+} and Mg^{2+} [7, 46]. In line with these observations, the specific activity of LipG7 was strongly enhanced by Mg^{2+} despite the lack of metal-binding sites on the predicted 3D structure. It is possible that Mg^{2+} enhances LipG7 activity by other mechanisms such as promoting a more rapid product release or cleaning the enzyme's active site as suggested by Voget et al. [47]. This hypothesis is corroborated by the fact that chelating agent, like EDTA, did not inhibit LipG7 activity, and discarding its's cofactor dependence.

Conclusion

Our study reports the first description of a recombinant esterase potentially from Porticoccaceae representative, identified in a mangrove sediment metagenomic library. Based on amino acid sequence homology and predicted structure analyses, LipG7 is a novel representative of the family VIII esterase with high homology to 1.4-butanediol diacrylate esterase of the *Porticoccus hydrocarbonoclasticus*, a highly specialized hydrocarbon-degrading bacterium, and also shares typical motifs of β-lactamases. LipG7 has outstanding esterase activity, emerging as an interesting candidate for biotechnological applications.

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Compliance with ethical standards

Conflict of interest The authors declared that they have no conflict of interest.

Ethics approval Collection of samples was registered by the pertinent environmental agency (A982E7D-SisGen).

Informed consent All co-authors have agreed to participate. All co-authors have agreed for this manuscript publication.

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